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Studies on the Morphology of the Nematode Heterakis Spumosa Schneider

STUDIES ON THE MORPHOLOGY OF THE NEMATODE HETERAKIS SPUMOSA SCHNEIDER

BY

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B. S. Knox College, 1919

THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF SCIENCE

IN ZOOLOGY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

1920

UNIVERSITY OF ILLINOIS

THE GRADUATE SCHOOL

June 1 1920

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Ellis Russell Weaver

ENTITLED STUDIES ON THE MORPHOLOGY OF THE NEMATODE HETERAKIS SPUMOSA SCHNEIDER

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science

In Charge of Thesis

Head of Department

Recommendation concurred in*

Committee on Final Examination*

*Required for doctor's degree but not for master's

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In the following paper, the writer has tried to give as clear and detailed description of the Nematode, Heterakis spumosa, as possible and takes this opportunity in expressing his most sincere thanks to Professor H. B. Ward for the suggestions and assistance which he has given.

STUDIES ON THE MORPHOLOGY OF THE NEMATODE
Heterakis spumosa Schneider

I Introduction

In 1845 Felix Dujardin formed a new genus of Nematodes which he termed Heterakis, meaning diverged spicules, with Ascaris vesicularis Froelich as the type. To this he also added three others, A. dispar Schrank, A. acuminata Schrank, and Fusaria brevicaudata Zeder, thus forming the original genus from four species. There was a further addition of species by Molin, Schneider, Drasche, Von Linstow and others, until, in 1913, as many as fifty had been included.

In 1866 Schneider gave a short description of a new species H. spumosa dealing briefly with the external appearance. No further work was published on this species until 1890, when Michele Stossich described this parasite in a manner similar to Schneider's previous description. In 1898 Stossich again added to the publications by a description of the external appearance of the female as well as the male.

Travassos, in 1913, added a new species H. psophiae, which, in his description, is very similar to H. spumosa except that the caudal end of the male of the former possesses ten pairs of papillae while that of the latter has nine, as was described by Schneider.

A further addition in regard to classification arose when Clayton Lane, in 1914, described a suckered round worm very similar to H. spumosa in internal appearance, but which he placed under a new genus and species, Ganguleterakis gangula.

Maurice C. Hall, 1916, gave a more detailed description of H. spumosa, including a little of the internal structure, and listed Ganguleterakis gangula Lane as a synonym.

Thus, there is much confusion in forming genera and species of parasitic nematodes, and not until detailed descriptions of the anatomies of the different species have been given, can a definite classification be accomplished. Although, in this paper, I have not attempted the latter, I have tried to give as detailed description as the limited time allowed, first, of the general appearance of the parasite H. spumosa, and, second, of the different systems excluding that of the nervous system.

II Material and Methods

The material for this work was obtained from the host Epimys rattus, which was gathered at Urbana, Illinois, the majority being caught in and around the University of Illinois dairy barns. Dissection was made as soon as possible after capture, but at times this was not soon enough to prevent freezing, and in this case, it was interesting to note that the parasites could withstand a varied temperature. Even though they would appear lifeless, in a short time after placing in tap water heated to a temperature of about 45° C., they would soon revive.

Forty-two wild rats, Epimys rattus, were dissected, and the parasite which is to be described found in all, with the exception of two immature males and the same number of immature females. The cause of these exceptions was undoubtedly the lack of time for infection. However, the species was found to be quite common and usually in large numbers in each host, varying from two to fifty-six. It was also interesting to note that with few exceptions, there was a much larger number of females than males. As an example of this, in one host from which 47 parasites were taken, only four were males. However, this was probably an extreme, for in others the females were not in such a great majority.

This particular species, Heterakis spumosa, was always found in the coecum and in the upper half of the large intestine and never was it found either above or below these limits. The

parasites were always free, either in the feces of the host, or on the walls of the coecum or intestine. They were sometimes in a straightened position, but more often coiled in a spiral or circle, or even in the form of an omega.

The removal of the worms from their natural habitat was indeed not difficult. A moistened camel's hair brush proved to be the most satisfactory, as this would in no way pierce the body wall. After taking them from the digestive tract of the rodent, they were placed in tap water at a temperature corresponding to that of the body of the host. As they soon collapsed if left in the tap water, they were killed and fixed as soon as possible. I have examined the adult specimen alive but the internal anatomy can be observed much better in those which have been fixed. However, the movements of the body are seen but these are very slow and for the larger part of the time there are none.

Technique. As a medium for fixation, glycerine and alcohol were used in a proportion of two parts of the former to twenty parts of the latter, of seventy percent grade. This medium was heated to a temperature of about 90° C., and poured over the parasites, from which practically all the tap water had previously been drained. This was done in order that the temperature of the alcohol and glycerine would be lowered as little as possible, and also that its strength would not be diluted. This method proved to be quite satisfactory, for at the high temperature used, the worms straightened and were instantly killed.

Also, there was no visible shrinkage of the body as could be noticed with the use of other materials. The specimens were allowed to stand in this fixative for any desired length of time, for since the presence of alcohol caused the bodies to become opaque, the evaporation, which was accomplished by allowing it to stand in the open atmosphere, left the specimens in dilute glycerine which gradually became stronger and acted as a clearing agent. This, however, did not make a transparency that was satisfactory, and to overcome this, they were taken from the glycerine and placed directly in lacto-phenol. This proved to be successful, that is when toto mounts were to be made. With artificial light the internal anatomy could be quite distinctly seen. This transfer from one medium to the other caused no shrinkage or collapse, providing the worm was allowed to stand in the glycerine and alcohol until the latter had practically all disappeared. Since the densities of lacto-phenol and glycerine are almost the same, there was no cause for collapse.

This, however, was very simple technique, much more simple than that in preparation for micratome sectioning. In the case of the latter the fixation medium consisted, as before, of glycerine and alcohol heated to the same temperature and similarly applied, but with an addition of a few drops of concentrated acetic acid. However, the evaporation of the alcohol was not allowed, as in the preparation for toto mounts, but instead, the specimen was transferred to alcohol of the same strength as that in which it had been fixed. From this it was slowly placed in higher

grades with steps of not more than two and one-half percent, and time intervals of not less than thirty minutes. In this manner, the worm was brought up to absolute alcohol and from there into pure xylol, by slowly increasing the amount of this clearing fluid. From this until the stain was applied to the slides, the technique was the usual simple technique of embedding. Parowax was found to be the most successful for this and was used in preference to other paraffines. The sections were usually made ten micra in thickness, as it was found that this gave more satisfaction than thinner sectioning.

Staining was accomplished on the slide more successfully than in toto, for in the latter condition I have not been able, in the limited time that has been taken, to find a suitable stain which would penetrate the cuticula. For this reason, all staining has been done after the serial sections are made. Transverse sections were rather difficult to mount, in that the bodies would not retain their original shape. However, this may be somewhat overcome if, when drying on the slide, the sections are heated almost to the melting point of the paraffine. This will cause the bodies to swell into their usual shape, but even then, oftentimes the cuticula will expand and raise from the underlying muscles.

Delafield's hematoxylin was used with a counter stain of eosin. The sections were usually overstained and destained with dilute acid alcohol. More differentiation could be obtained in this manner than with the few other stains that were tried.

Damar was used as a mounting medium. On account of the small size of this species, it was necessary to use high magnification, oftentimes an oil immersion lens only serving the desired purpose.

III General Appearance

The external appearance of Heterakis spumosa is that of Nemathelminthes in general, that is, cylindrical, rather elongate, both the anterior and posterior ends somewhat pointed, and most important of all, a lack of segmentation. The male and female do not have a striking difference in size, but, all in all, there is a great variation in length and thickness in different individuals. In all my observations I did not see a worm over 10.29 mm. long, but the range showed many variations in length, depending upon the maturity. (See tabulation). The one of the largest size was a female and exceeded any male by 0.71 mm. However, in taking an average of the lengths of fourteen individuals, six males and eight females, all of which were matured, I found the average length of the male to be 8.31 mm., while that of the female was 8.308 mm. From this I concluded that on the average there is practically no difference in the lengths according to the sex. The maximum diameter of the body, taken at the junction of the intestine and oesophagus, was that of a female also, which measured 0.309 mm., exceeding any male by 0.027 mm. The average diameter of the same individuals as those measured for length, was 0.2435 mm., the average of the males being 0.239 mm., while that of the females was 0.248 mm. These measurements clearly show that the female has the larger diameter, both in the average and in the maximum measurement.

Sex	Length of worm	Vulva to posterior end	Oesophageal bulb to posterior end	Anus to posterior end	Width at anus	Width at vulva	Width at oesophageal bulb	Width at mouth
Female	6.016	2.744	0.846	0.309	0.094	0.188	0.206	0.075
Female	6.392	3.158	0.804	0.556	0.094	0.188	0.206	0.075
Female	7.44	3.496	0.836	0.62	0.112	0.244	0.206	0.075
Female	7.65	3.609	0.827	0.606	0.126	0.288	0.246	0.078
Female	8.36	3.76	0.94	0.658	0.131	0.288	0.309	0.094
Female	9.713	4.60	0.846	0.676	0.121	0.290	0.282	0.094
Female	9.95	4.78	0.94	0.676	0.1128	0.319	0.282	0.112
Female	10.29	5.33	0.94	0.77	0.169	0.319	0.244	0.094
Male	6.392		0.752	0.282	0.094		0.188	0.075
Male	6.392		0.808	0.288	0.188		0.210	0.066
Male	8.27		0.94	0.309	0.225		0.263	0.094
Male	8.648		0.95	0.282	0.225		0.244	0.075
Male	8.678		0.995	0.309	0.188		0.282	0.094
Male	9.585		0.902	0.282	0.225		0.282	0.094

Cuticula. The cuticula which covers the body of Heterakis spumosa shows fine transverse striations, about 24 to 0.01 mm., and in living specimens is somewhat semi-transparent, although at the same time giving a white appearance. The transparency is only brought out through the use of the microscope and a strong artificial light. Specimens which have been killed in hot alcohol become opaque, due in all probability to the coagulation of the protoplasm in the muscle cells. This cuticula, when thus treated with alcohol, also becomes rigid and hard, while in the living animals there does not seem to be such a rigidity, but instead it is more flexible.

Anterior End. The anterior end of both sexes tapers rather abruptly to an average diameter of 0.094 mm., while the posterior end differs in the male from that of the female. With the latter there is a more gradual tapering to a sharp point than that mentioned in regard to the anterior end. With reference to the male there is a very abrupt decrease in diameter of the body posterior to the cloacal opening and paracloacal papillae. From this point to the tip of the tail there is a gradual decrease, ending in a sharp point, the same as with the female.

In both sexes the anterior half of the body gives the same general external appearance and can thus be described. The mouth is surrounded by three distinct, subequal lips, (Fig. 5), each bearing two papillae which may be called lip papillae. These are very small conical outgrowths of the cuticula with a diameter

at the base of 0.0188 mm. and a similar height of extension above the external surface of the body. The extremity ends somewhat bluntly.

Posterior to the mouth opening, in a mature individual a distance of about 0.16 mm., there is an abrupt outgrowth of the cuticula on either side of the body in the form of a membrane, attached to the cuticula by one of its very thin edges. This arises, as has been stated, rather abruptly and reaches its greatest development (Fig. 1) in the region of the excretory pore about 0.372 mm. from the anterior end. Posterior to this point, there is a gradual decrease in the development of this lateral membrane until, in the region of the oesophageal bulb, it reaches a width of 0.012 mm. In the case of the female there is then a constancy in the protrudance of the membrane or alae to the region of the anal opening where it slowly decreases but does not entirely disappear until at the tip of the tail. With regard to the male there is a very contrasting difference at the caudal end which the writer will describe later in this work.

The lateral alae also shows the characteristic transverse striations that are seen on the body covering and also possesses a semi-transparency as does the cuticula.

On the anterior end of either sex of this parasite, 0.04 mm. from the mouth, are two papillae, one on either side and situated in the median lateral line. They are quite broad at the base, having a diameter of 0.012 mm. and rise to a height of 0.006 mm. They end rather bluntly and appear to be formed from the

sub-cuticular layer, differing in this respect from the lip papillae which seem to be only swellings of the cuticula. They have not been seen to pierce the body covering.

Situated at the points where the lateral alae begin, there are two blunt protrusions of the sub-cuticular layer, but contrasting from those papillae previously described in that they have a very large basal diameter, averaging about 0.03 mm. with a height of 0.004 mm.

Posterior to this pair, approximately 0.408 mm. from the anterior end, is still another pair of papillae, situated in the lateral alae at the point of its greatest development and in the region of the excretory pore. These may be described as conical in shape also, rising from the sub-cuticular layer and lying in the lateral alae. They have a diameter at their junctions with the body wall of 0.012 mm. and rise to a sharp point 0.024 mm. from their bases. These are termed by Hall as the cervical papillae.

The external appearances that have already been described are the same in both sexes, but posterior to the oesophageal bulb differences are found, thus making a separate description of each sex necessary.

Slightly posterior to the middle of the body of the female is the genital aperture. This consists of an opening with two rather large transverse lips which rise 0.056 mm. perpendicularly above the body wall, and each of which is 0.047 mm. in thickness. Either before or behind this vulva is a cuticular

prominance, in some specimens only barely visible, in others rising 0.024 mm. from its base, while in other individuals they are as elongate as the lips of the genital opening. (Fig. 6).

Posterior to the vulva, the cuticula remains smooth except for the fine striations and lateral alae, which have previously been mentioned, until the anal opening is reached. This is usually about 0.6580 mm. from the caudal tip and marks the point of abrupt decrease in the diameter of the body. Situated in each median lateral line 0.386 mm. posterior to the anus is a pair of small conical papilla comparing quite favorably in shape and size to the cervical papillae previously described.

Posterior End. Not until in the posterior third of the body of the male are there any eruptions or markings in the cuticula. It is this part of the body that distinguishes the sex at the first glance and is the distinguishing characteristic of the species.

A well developed sucker is located in the mid-ventral line of the body 0.564 mm. anterior to the caudal end. This organ consists of a stalk or peduncle approximately 0.047 mm. long, joined to the body wall, and at the opposite extremity of which is a heavy chitinous ring, elliptical in shape and transversely of the body. The appearance given is that of a small rubber tube which has been slightly turned back upon itself. The average size of this elipse in adult specimens is 0.0564 mm. long by 0.1034 mm. wide. The well developed muscles of this pedunculate structure can be seen through the cuticula

and appear to extend to the outer extremity. The chitinous ring which has already been mentioned is interrupted on its posterior border by an elevation somewhat triangular in shape with the apex pointing caudad.

A bursa which is entirely of the same substance as the lateral alae arises abruptly on either side of this posterior portion, beginning about 0.25 mm. anterior to the sucker. It consists of two thin transparent layers on each side which extend at about a 45° angle latero-ventral to the body wall of the animal. In their greatest development they rise to a thickness of about 0.20 mm., which is opposite the anal opening and is 0.319 mm. from their abrupt beginning. They then decrease more abruptly than they began and at the junction of the body with the tail, which is 0.188 mm. from the posterior end, they are again about the thickness of the cuticula over the body proper. As has been stated, this bursa is of two layers, the one nearer the sucker being somewhat smaller and decreasing toward the tail in a more gradual manner. Also in this layer, caudad of the sucker, there is a deep depression dipping almost halfway to the body. The second layer, the one farthest from the sucker, rises to a greater development and shows a more abrupt decrease at the junction of the tail and the body than the one just described.

The bursa is sustained in the region of the anus by three pairs of large papillae. Of these the one most anterior is the largest and seems to be quite granular in its appearance. This pair is flattened dorso-ventrally and the papillae are

0.0658 mm. wide at the base in the average mature adult. The sides of the papillae rise perpendicularly to the body wall for 0.0658 mm. where they suddenly decrease to a small round tip 0.0188 mm. in diameter and 0.0188 mm. long. In all, the papilla rises 0.0846 mm. above the cuticula.

The next pair, median between the anterior and posterior, is about half as large as the first of this group, but is practically of the same shape and structure. It also is possessed with the small tip and is flattened dorso-ventrally. However, the entire height of this papilla is 0.0366 mm. with a basal width of usually about 0.028 mm.

The posterior pair of papillae is situated in a position similar to the two pairs already mentioned, but differ in appearance, inasmuch as each papilla is cylindrical in shape with a diameter of 0.0188 mm. and are somewhat longer than each of the median pair, the length corresponding more nearly to that of the anterior pair, which was stated as 0.0658 mm. The diameter is rather constant until at the outer end it decreases slowly to a blunt ending.

In addition to the three pairs of papillae already mentioned, there are two small pairs found on either side and both posterior and anterior to the anal opening. The hindermost pair is located close to the side of the anal aperture while the remaining pair is anterior to the anal opening and even to the largest pair sustaining the bursa. These papillae are quite small having a diameter and length of 0.0188 mm. and end real bluntly. (Fig. 2).

Still anterior to these and on either side of the sucker are two more pairs. These, however, are very much longer and appear more nearly like the posterior pair of bursal papillae in shape and size. They seem to come from near the base of the sucker and project outwards from the body wall making an angle with the peduncle of about 30 degrees. They are cylindrical in shape and stand higher than the sucker. They have a rather constant diameter of 0.0188 mm. and end, as do the other caudal papillae, real bluntly, oftentimes possessing a small knob.

The bursa does not extend over the tail region, thus allowing the cuticula to form the smooth covering characteristic of the body. However, about 0.075 mm. from the anterior end, three pairs of small papillae arise from the sub-cuticular region and attain a length of 0.028 mm. They are on either side and grouped close together, the three lying in a space of 0.056 mm. They are cylindrical in form, having a constant diameter throughout their entire length of 0.009 mm. Their average length is 0.028 mm. From this point the diameter of the tail region rapidly decreases in the adult and ends in a sharp point about 0.188 mm. from its junction with the main part of the body.

In observing the immature specimens, the writer notices that in either sex there is a tendency for a narrower body in comparison with the length, and that as the animal grows older there is a tendency for the body to shorten and thicken, comparatively. The increases and decreases in the diameter are noticeably more gradual in the young individual than in the adult.

IV General Anatomy

Body-wall. The cuticula which covers the body of Heterakis spumosa is very resistant and is composed of two layers, an outer one which is quite thin and an inner layer which appears much thicker and is less resistant. No fine striation appears in this as is seen on the outer layer. The two layers taken together differ in thickness in different regions of the body. At the anterior end and in the cephalic region it is 0.0047 mm. thick, being thinner than at any other part of the body. Near the median part it increases to 0.0188 mm. and gradually does so until at the posterior end in the anal region it has a thickness of 0.0376 mm. The two layers compare favorably in the change of thickness over the entire body, the outer one being about one-third that of the interior layer. There is undoubtedly a chemical difference in the composition of the two, for the inner will take the stain to a greater extent, in fact the outer layer will resist the stain altogether. This of course distinctly shows the line of separation. Beneath this there is a very thin sub-cuticular layer to which the basal portions of the longitudinal muscles are attached. In the median lateral lines, this hypoderm or sub-cuticula extends into the body cavity forming the lateral bands of which more will be stated later. Also, in the female this layer protrudes into the body cavity in the mid dorsal and ventral lines, thus forming the dorsal and ventral bands. (Fig. 9).

The lateral alae of both sexes and the bursa of the male are of the same composition as that of the cuticula, but seem to be a product of the latter, for the cuticula can be seen to pass between the musclature layer and the bases of the bursa or alae. The staining properties of the latter seem to be almost as great as that of the muscles.

There is a particular hardness noted in the cuticula and especially in killed specimens and as there is no cell structure or nuclei present, it must be a chitinous secretion.

At the oral and anal apertures, the cuticula folds in and lines the oesophagus and lower intestine respectively. More, however, will be given of this under the description of the digestive tract.

On either side of the body, beginning in the cervical region as small cavities and running caudad parallel to the oesophagus, are two lateral bands or lines. These extend in the female to the region posterior to the anus and differ in size and structure according to the part of the body that is examined. The male here differs in that the posterior end of the lateral bands is in the region of the sucker. At the anterior end in the region of the mouth, no traces of the lateral lines can be found, but in the cervical region small cavities appear and gradually increase in size until near the excretory pore the size is maintained to the posterior end. At this region the cavity seems to be filled with a granular substance and increase in height, pushing out into the body cavity, seemingly to serve as a support for the alimentary

tract. At a point slightly posterior to the excretory pore, the excretory tubule which runs on either side of the body enters the lateral line and continues to the anal region. There are no distinct changes throughout their courses except that in both sexes they increase in size in the anal region and occupy almost half the body cavity. In all the observations made, the writer has found that in the female the lateral lines are low and broad, while in the male they are more narrow and rise to a greater height. A division appears in the lateral bands in the region of the oesophageal bulb and persists throughout the entire length. It seems to consist of two walls giving the appearance that each half has a wall of its own. It is between these two walls that the excretory tubules usually push their way. However, they do not always stay within these limits, but sometimes lie quite free from it. The nuclei are very numerous especially in the anterior end and appear to lie in the wall around the cavity.

On the mid-dorsal and ventral sides of the female there are also dorsal and ventral bands. Their origin appears to be in the region just anterior to the vulva and their disappearance at the anus. These are very much smaller than the lateral bands which have previously been described and appear almost round. At first appearance they are very minute but gradually increase in size until at their posterior extremities they are half as large as the lateral bands, a diameter at this point of approximately 0.094 mm. They are also filled with a granular mass but do not push out into the body cavity as far as those on the sides. How-

ever, they do extend, in the posterior regions, far enough to separate the processes of the muscles. At their posterior ends, they seem to gradually disappear and small muscle fibres to take their place.

Neither have been found in the transverse sections made of the male and for this reason it is the belief of the writer that they do not exist. One is able to see a division of the muscle fibres in the region where they should be found, but no dorsal or ventral bands have been seen.

Muscular System. In the cross sections made of Heterakis spumosa, I have been able to observe four rows of muscles, the four divisions being caused by the lateral, dorsal, and ventral lines. However, this is a characteristic of all Nematodes and is not peculiar to this genus or species alone.

The musculature of the two sexes is practically the same, although there are some differences which will be taken up later in this description. However, that with which the writer will deal at present is common of both male and female.

The muscular system consists of spindle-shaped fibres rather long and somewhat narrow and arranged longitudinally of the body. Although differing in different regions, one will find an average of twelve or more fibres arranged side by side in each quadrant. They differ greatly in size and therefore measurements would be of little value. The external edges of the fibres are in contact with the inner side of the sub-cuticula and on the opposite edges fine processes are sent out into the body cavity.

These processes extend oftentimes as far as and seem to unite with the wall of the digestive tract. They in no way connect with the lateral bands, but instead, seem to diverge from them. In the foremost region of the body, anterior to the excretory pore, these muscular processes are very few in number and the muscular fibres are rather broad. The four divisions previously mentioned are not shown, as the lateral, dorsal, and ventral bands are not present.

The cephalo-oesophageal muscle fibres are very much narrower and are possessed of a large number of processes, some of which seemingly are inserted into the oesophageal wall. Others are shorter and only extend into the body cavity. In this region there is a distinct division into four quadrants which continue throughout the remainder of the body. The processes are not only more numerous but many of these are branched, thus making a network of muscular fibres throughout the body wall. Usually they are connected, one to another.

In the posterior region of the male, the muscular system becomes a little more complex, caused by the presence of the sucker. Here the muscle fibres become very much narrower and a greater number of processes extend from them. Although no dorsal or ventral lines are present, there is still the tendency to divide into four regions.

In the region of the sucker, the muscle fibres turn from running longitudinally in the body and extend ventrally into this organ, although lying in the same position as in the body

proper. The writer has observed no processes sent out from the fibres inside the sucker. The fibres are here rather thick and well developed. They end quite abruptly in the ring of the sucker. Posterior to the anal aperture, the muscle fibres decrease until in the tip of the tail there appears to be only a few bands.

In the posterior end of the female the arrangement of the muscles is as has been described for the anterior part of the body. The fibres are divided into the four characteristic regions by the dorsal, ventral and lateral bands, but the number of fibres decreases immediately upon entrance into the tail. This decrease continues until as in the male there remains only a few muscular fibres. In contrast to the condition in the male, the process from the muscular fibres are very few and short. In the region of the vulva there is in addition to the regular muscular band, a heavy undivided wall around the vagina. This is unlike the characteristic musculature in that it is not divided into fibres. The musculature of the oesophagus consists of two kinds of muscles which are distinctly shown by their ability to take a stain. The first to be mentioned are those that begin in the sides of the triradiate lumen and extend to the margin of the oesophagus. These muscles are found in greater numbers than the second kind, which run from the tips of the lumen to the periphery. The latter muscles have much greater staining properties and always appear in the form of bundles. They are found in the entire length of the oesophageal bulb. The anal muscle is also different in that it consists of a one-celled muscle encircling the anal

opening. It has been designated as the anal sphincter muscle.

The body cavity is quite limited and small, inasmuch as it is almost filled with the organs of digestion and reproduction. In it there is a granular mass which seems to have no definite form other than the granules seem to be more concentrated around the muscular processes and around the intestinal wall. At the junction of the oesophagus and intestine are found large granular cells, with a diameter of approximately 0.024 mm., which encircle the digestive tract. The cells are apparently not connected as no connection to the alimentary canal has been observed. In cross section each cell is composed of a granular mass and a large flattened nucleus. Since the fluid of the body cavity around these cells is more granular than elsewhere, it is the opinion of the writer that their function is the secretion of the body fluid.

Digestive system. The digestive tract is divided into a mouth capsule extending back from the oral aperture, usually in the adult worm 0.04 mm., an oesophagus extending from this point for a distance of about 0.88 mm., and an intestine connecting the oesophagus with the anal opening. Other minor divisions will be given later.

The mouth capsule opens on the anterior end and is lined with a cuticular layer which is merely an infolding of the body covering and, therefore, has the same appearance. There are the same fine transverse striations that have been mentioned as characteristic of the external layer. At the margin of the mouth

are two small chitinous tooth-like structures partially embedded in the capsule wall. (Fig. 4). These structures are rather broad at their bases or points of muscular attachment, 0.005 mm., and present a hooked appearance, growing horizontally toward the lips and piercing the cuticular lining at the anterior end of the capsule. They end as pointed structures extending into the mouth cavity from the cuticula only a short distance, varying in different individuals, and lie on either side, pointing toward each other. At the basal ends, attachment is made by a small longitudinal muscle band extending perhaps, as an average, 0.005 mm. before joining the muscular bands of the body wall.

The writer believes these bodies serve the purpose of teeth, which is true of numerous other species of Nematodes.

The wall of the mouth capsule is quite muscular and the lumen has the shape of a three-angled figure in transverse sections, with the sides pressed toward the center, giving the appearance of a collapsed isosceles triangle. There is no apparent increase or decrease in the size of the capsule or lumen until at the junction with the oesophagus.

At this point there are a number of changes that are, indeed, important. But before further detailed descriptions, I will endeavor to give an account of the general appearance of the oesophagus. It is divided into two regions, an anterior cylindrical portion a trifle larger in diameter than that of the mouth capsule, and a posterior pear-shaped bulb. With the two parts taken together, the length is approximately 0.752 mm., while the

diameter of the anterior portion is 0.047. That of the posterior part varies, on account of the shape, from 0.0752 to 0.1504 mm. However, the internal structures are quite important.

The passage of the lumen of the mouth to that of the oesophagus is made possible by the same chitinous lining as is found in the mouth capsule, but the triradiate lumen of the latter swells out into a somewhat funnel-shaped tube on its entrance into the oesophagus. This funnel, if it may be so called, decreases in size posteriorly, perhaps 0.011 mm. from the anterior beginning, to a lumen similar in size and shape as that of the mouth capsule. Three small ducts lying in the thick muscular wall of the oesophagus and coming from a hinder region, open through as many apertures into the funnel. (Fig. 4).

From the posterior region of the funnel, the lumen continues with the same size and appearance to the oesophageal valve in the larger part of the oesophageal bulb. Here the canal increases in size about two-fold, although still keeping its characteristic shape, for a distance of about 0.02 mm. In the adult specimen, usually about 0.8 mm. from the junction of the intestine and oesophagus, are six small conical outgrowths projecting into the lumen in such a manner that when pressed together they can form a temporary division or valve. (Fig. 9). The musculature of this valve has been dealt with at an earlier point in the paper. Again the lumen becomes the normal size after the valve is passed and so continues to the posterior end of the oesophagus.

Throughout the heavy muscular wall of the entire bulbous region, glandular structures are found. In the observations of

the writer there has usually been three of these structures extending from a blind ending in the hindermost part of the intestine to the anterior end of the oesophageal bulb. They are situated radially, each gland usually between the radial extensions of the lumen. However, there may be exceptions to the latter, as in some specimens branches appear to be given off, thus increasing the number. In such case, they are found around the tips of the lumen as well as between them. Their shape is somewhat varied, but in most cases, appear in transverse sections as triangular with the apex pointing toward the lumen. As has already been mentioned, the three main glands, with no reference to the branches, extend the length of the bulb, larger at the posterior and gradually decreasing until at their foremost ends they suddenly disappear. They are, however, connected from this point by three small ducts, also running in the wall of the oesophagus, to the funnel at the junction of mouth capsule. Here, they change their course, which has been longitudinal to the body, and extend transversely of the oesophageal wall, opening into the cavity of the funnel. (Fig. 9). Near the posterior end of these glands there are definite connections one to the other at the interior edge and very close to the lumen.

The intestine begins with much the same size and shape as the oesophagus ends. It has a diameter at its foremost region of 0.135 mm., decreasing in a space of 0.62 mm. to a diameter of 0.067 mm., which is constant until near the anal region there is a sudden increase. This larger posterior portion may be termed

the rectum and will be described later in this paper. The other portions will be taken up separately.

The lumen of the anterior part of the intestine is merely a slit rather than triradiate, as was that of the mouth capsule and oesophagus, and is surrounded by a cuticular layer similar to that of the digestive tract which has previously been described, except that it is somewhat thinner. The size of the lumen in this region is 0.075 mm. long, while the walls may be separated perhaps 0.02 mm. The latter varies, however, and cannot be given as a true measurement.

The intestinal wall is, in the foremost division, extremely thick, usually about 0.057 mm. with heavy muscular layer around the canal, but having a more granular appearance nearer the exterior wall. Although in cross section no definite cell walls have been seen, there are large nuclei present, ranging from four to ten in number. From this the writer is able to say the cells are unusually large and that, by the arrangement of these nuclei, the wall is only one cell in thickness.

Throughout that part of the intestine in which there is a constant diameter, and which has been termed by several authors as the chyle intestine, the lumen is somewhat branched and is surrounded by a thin cuticular layer. The walls are comparatively thick, but are not as muscular as the portion that has just been described. The cell structure is also the same, the nuclei being large and few in number.

Anterior to the anal opening for a distance of about

0.13 mm. the alimentary tract which is called the rectum, bears a larger diameter than that of the chyle intestine and contains a lumen, which, instead of being a narrow slit, is rather large and is ovoid in shape. In both sexes it gradually flattens dorso-ventrally until at the anus it is very narrow and has a length of 0.046 mm. Thus, the anal opening appears as a slit of this size and transversely of the body. The wall of the lumen consists of a thick cuticular layer very much like that of the body covering or the lining of the mouth capsule. The muscular layer consists of a single cell having a thickness varying from 0.011 mm. at the anal aperture to 0.018 mm. at the most anterior part of the rectum.

Reproductive system. The reproductive system of the female consists of a vagina, which is a single tube, uteri, and ovaries, each of which will be described separately.

The external aperture of the genital glands, the vulva, is situated a little posterior to the median part of the body and has already been described. The vagina, which consists of a single tube, immediately at its connection with the vulva, turns cephalad for 0.13 mm. where it sharply turns caudad for 0.33 mm. and divides into two uteri. The tube has a constant diameter of 0.036 mm. and, in external appearance, seems to be surrounded by a spiral band. (Fig. 10). The wall is extremely thick and muscular and the lumen varies in form from that of quadradiate at the anterior end to a mere slit at its junction with the

uteri. This is, however, expanded or contracted according to the number of eggs contained. The uteri consist of two opposing tubes, one continuing from the vagina toward the posterior end and the other turning toward the anterior end. Either uterus has a diameter of 0.04 mm. and contains a lumen which is almost round and has a diameter of 0.018 mm. The wall is quite muscular and consists of a number of cells, although never more than one cell in thickness.

The two uteri are finally transferred into ovaries which coil back and forth in the adult specimen, filling the body cavity. The ovaries consist of two rather large thin-walled tubes varying in diameter and containing a granular mass in which are scattered large nuclei, presumably the nuclei of eggs in their early stages. The egg when deposited is ovoid in shape and has a length of 0.059 mm. with a width of 0.048 mm. At this time it is also surrounded by a shell having a thickness of approximately 0.0027 mm.

The reproductive organs of the male consist of the cloacal opening to which is joined the seminal vesicle, a small tube or duct leading from the seminal vesicle at one end and a testicular tube to which it is attached at the opposite extremity. The seminal vesicle lies immediately anterior to the cloacal opening and consists of a large tube, with a diameter varying from 0.056 mm. to 0.094 mm. and a length of about 0.752 mm. in the average specimen. It suddenly increases in size from the cloacal region and reaches its greatest size, 0.282 mm., anteriad. It then gradually decreases in size until it joins the

seminal duct which is 0.056 mm. in diameter. The walls are approximately 0.01 mm. thick and are made up of large cells. The writer has found it rather difficult to see the nuclei of these cells, since they are very scattering. They are large and somewhat oval in shape. The lumen varies according to the diameter of the vesicle, but since the thickness of the walls is fairly constant the size of the lumen is quite easily determined.

The seminal duct is a small tube with exceedingly thick walls in comparison to the diameter and with a slit-shaped lumen. The structure of the muscular wall, unlike that of the seminal vesicle, is of numerous small cells whose nuclei are plainly seen. The wall is, however, only one cell in thickness. The duct has an almost constant diameter of 0.056 mm. and a length of 0.47 mm.

The testicular tube is larger in diameter than that of the seminal duct at their junction and which at this point contains a large lumen. However, there is a rapid decrease in size as the tubule winds anteriad, and the lumen is gradually filled with a granular mass, presumably containing spermatozoa. This single tube winds and coils about the intestine as far forward as the oesophageal bulb where it ends blindly.

The spicular apparatus of Heterakis spumosa consists of two sub-equal elongate and narrow bodies lying longitudinally in the muscular wall anterior and dorsal to the cloacal opening. In transverse sections they appear as squares with somewhat

rounded corners and increase in size at the basal end, or the one most anterior. At this point they are in the average adult 0.0216 mm. thick. Toward the opposite end which is curved ventrally, the spicules gradually become pointed, ending shortly below the cloacal opening. Their entire length is 0.25 mm. Each is inclosed in a sheath which appears to have no cell structure and which has only one opening, it being at the posterior end. This sheath has been termed by Looss and others as the gubernaculum. At the base of this there appears a small hook-like process (Fig. 3), to which muscle bands are attached. Also the entire length of the spicules seem to be inclosed in a muscular sheath. The use of this sheath will be explained later in this paper.

The internal structure of these spicules, in transverse sections, seem to consist of a somewhat granular mass which stains very easily and deeply. The author has been able to see only one nucleus in each spicule, this being real large and located near the pointed end.

During copulation the spicules are ejected by means of the surrounding muscle sheath along their sides contracting and thus pushing them out. On being forced out in this manner, the base of the gubernaculum is also pulled out along with the spicule, being turned in upon itself in the operation. By retracting, the muscles attached to the hook-like process at the base of the spicule are able to pull the latter back into the sheath. Although this has not been actually seen in copulation, by the arrangement and attachment, this method of operation is the opinion

which I have drawn.

Excretory system. The excretory function is given over to two small tubules lying on either side of the body and extending from the anal region to a median ventral pore located about 0.372 mm. from the anterior end. This pore is very minute and opens into a single short tube which extends dorsally and toward the digestive tract. However, this divides into two smaller tubules which at first tend to lie on the ventral side of the oesophagus. In this position and in the region median between the excretory pore and the beginning of the oesophageal bulb, these small excretory tubules seem to penetrate two large granular structures. I have been unable to determine the function of these glands, but in location and appearance they compare quite favorably with those glands which were called by Looss as the cervical glands.

Shortly after leaving these structures, the excretory tubes diverge and enter the lateral bands. Here they continue toward the caudal end, gradually growing smaller and smaller until near the anal region they appear to end blindly. The shape of the tubules is round except in those places where they are pressed by surrounding organs. The walls are very thin and take the stain quite readily.

V Conclusions

The following points are those which have not been previously described for Heterakis spumosa:

1. There are two pairs of lateral papillae located between the lip papillae and the cervical papillae.

2. The cuticula is composed of two layers.

3. There are sphincter muscles at the vulva and anal orifice.

4. There are two tooth-like structures located on the interior wall of the mouth capsule.

5. An oesophageal funnel is found at the junction of the mouth capsule and the oesophagus.

6. The walls of the bulb of the oesophagus contain oesophageal glands which are connected with the oesophageal funnel by three small ducts.

7. An oesophageal valve is found in the bulb of the oesophagus.

8. The spicules are not flattened as described by Hall but are quadrilateral in cross section.

9. The excretory tubules are located in the lateral bands caudad to the anterior part of the oesophageal glands.

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VII Explanation of Plate

Anus	a.	Oesophageal gland	oes.g.
Bursal lobe	b.l.	Oesophageal gland duct.	oes.g.d.
Bursal papillae	b.pap.	Oesophageal valve	oes.v.
Cervical papillae	c.pap.	Para-cloacal papillae	p.pap.
Cuticula	cu.	Papillae	pap.
Excretory duct	e.d.	Spicules	sp.
Excretory tubule	e.t.	Sucker	s.
Lateral alae	l.a.	Tail Papillae	t.pap.
Lateral bands	l.b.	Tooth	t.
Nucleus	n.	Uterus	u.
Oesophagus	oes.	Vagina	va.
Oesophageal funnel	oes.f.	Vulva	v.

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Fig. 1. Ventral view of the anterior end of either sex showing outline of oesophagus. X 64.

Fig. 2. Ventral view of the caudal end of the male. X 64.

Fig. 3. Lateral view of the caudal end of the male. X 64.

Fig. 4. Anterior end of either sex. X 446.

Fig. 5. Anterior end of either sex showing lips. X 446.

Fig. 6. Lateral view of vagina. X 446.

Fig. 7. Cross section taken 0.5 mm. from ant. end. X 306.

Fig. 8. Cross section taken in region of excretory pore. X 306.

Fig. 9. Cross section taken in region of oesophageal valve. X 218.

Fig. 10. Cross section taken in region of vulva. X 218.

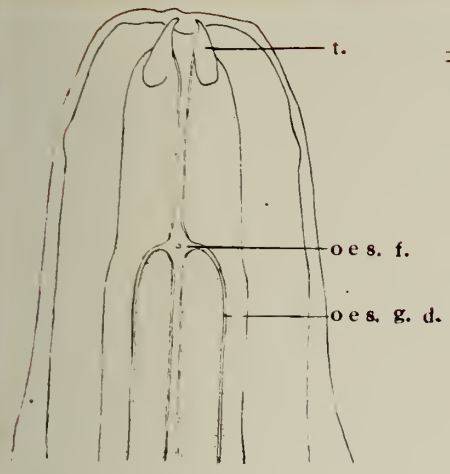

fig. 4

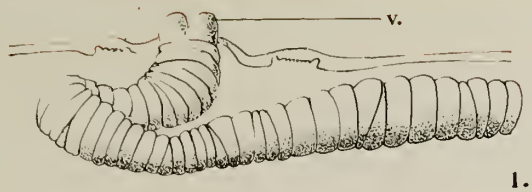

fig. 6

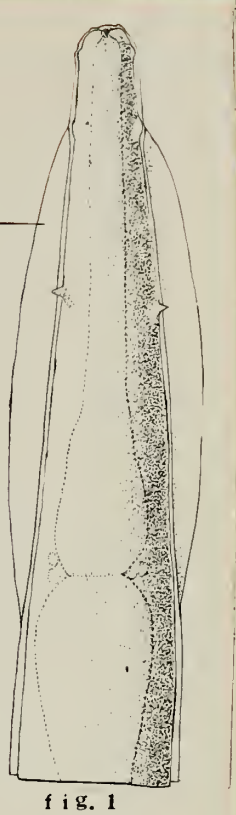

fig. 1

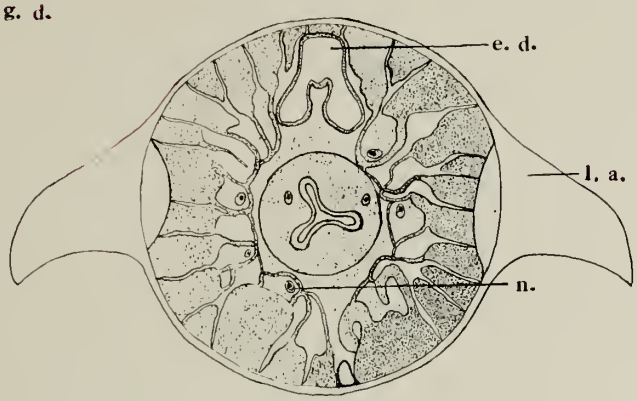

fig. 8

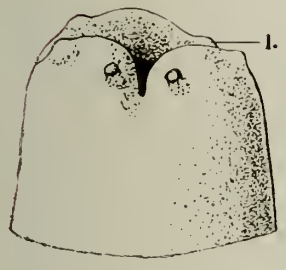

fig. 5

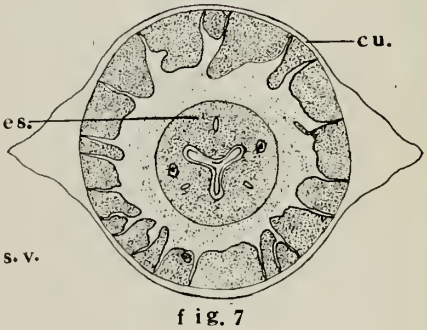

fig. 7

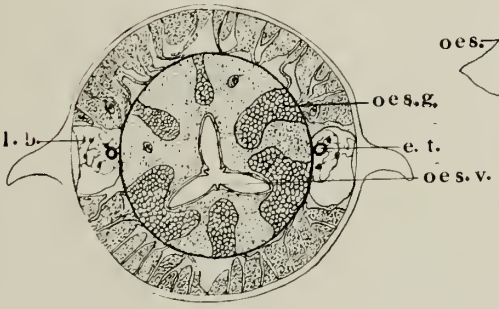

fig. 9

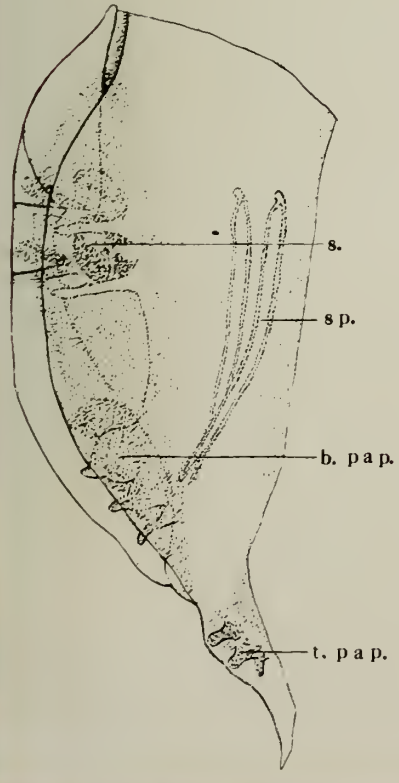

fig. 3

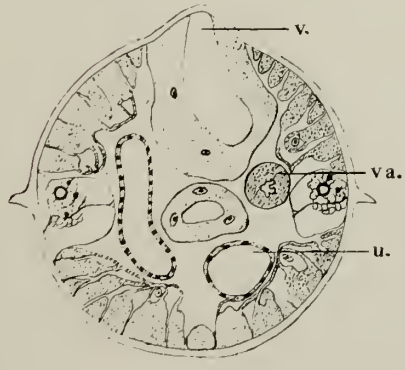

fig. 10

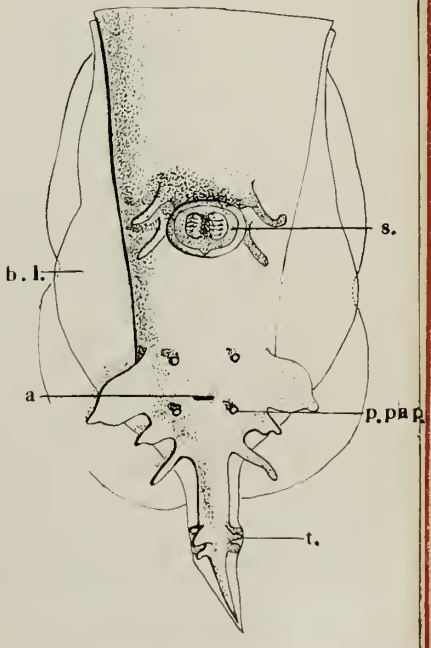

fig. 2